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Antiproliferative Effect of *Raphanus sativus* L. var. *caudatus* Alef in Human Breast MCF-7 and Human Lung SK-LU1 Adenocarcinoma Cell Lines

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Abstract

Introduction: This study was aimed to investigate the *in vitro* antiproliferative effect of Thai rat-tail radish—*Raphanus sativus* L. var. *caudatus* Alef or RS—the Thai indigenous plant in the same family as broccoli and cabbage. The isothiocyanates (ITCs) have been well-pronounced as the chemopreventive compound found in Brassicaceae plant. **Methods:** In this study, pod and stem of RS were extracted by dichloromethane via liquid-liquid extraction. Pure ITCs compounds—sulforaphane and sulforaphene—were used as the standards and positive controls. The crude extracts were inve stigated for their antiproliferation assay in the human lung cancer (SK-LU1) and human breast cancer (MCF-7) in comparable with the normal Vero cell line. **Results:** The results showed that only extract of RS pod showed antiproliferative effect. The SK-LU1 cells were most sensitive to sulforaphane (IC_{50} = 8.10±0.62 µg/ml), sulforaphene (IC_{50} = 17.05±0.18 µg/ml) and the RS pods extract (IC_{50} = 84.22±2.39 µg/ml) than those in MCF-7 cells. **Conclusion:** The present study indicated the potential of RS for further extended study of the chemopreventive effect of RS.

Keywords: Cytotoxicity; Sulforaphane; Sulforaphene; Isothiocyanates; *Raphanus sativus* L. var. *caudatus* Alef

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1. Introduction

The natural chemoprevention is the action of the natural compound on the basis of the cancer cell death-inducing activity (Machana *et al*., 2012). The target mechanism of chemopre ventive activity is a programmed cell death or apoptosis induction which indicates the pharma codynamic endpoint (Sun *et al*., 2004; Pocasap *et al*., 2013).Lung cancer is found in the high incidence worldwide, and breast cancer is also critically concerned among woman cancer. The discovery of compounds with apoptosis-induced cell death has been intensively explored which were from plants, edible vegetables and fruits (Cragg and Newman, 2005). Brassicaceae such as broccoli, cabbage, radish, and wasabi were reported to be the potential plant source of compounds possessing health promoting benefits (Jahangir *et al*., 2009; Björkman *et al*., 2011). Their function are not only attributed to fibers, vitamins, and minerals but also the secondary metabolites such as polyphenols, glucosinolate sand isothiocyanates (Jahangir *et al*., 2009; Björk man *et al*., 2011; Cartea *et al*., 2011) which were chemoprevention promised and prevented several degenerative diseases such as cardiovascular disease and diabetes (Cavell *et al*., 2011). The anticancer mechanism of action of well-known isothiocyanate, sulforaphane was the induction of programmed cell death or through apoptosis pathway (Kristjansdottir *et al*., 2012). Therefore, in this study, the Thai native plant in Brassi caceae family—*Raphanus sativus* L. var. *cauda tus* Alef or Thai rat-tailed radish—was determined for its chemopreventive effect against two commonly found cancerous cell lines in comparison to the normal cell line.

2. Material and Methods

Materials

The analytical grade dichloromethane was purchased from Fisher scientific, UK. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from GIBCO (Life technologies, California, USA). Neutral red dye was purchased from Sigma Chemicals Co. (Missouri, USA). L-sulforaphene was purchased from Enzo LifeScience (NY, USA). D,L-Sulforaphane was purchased from Calbio chem (Merck, Darmstadt, Germany).

Sample preparation and extraction

Thai rat-tail radish or *Raphanus sativus* L. var. *caudatus* Alef (RS) was harvested at the 6^{th} weeks. Fresh stem and pod parts were used for the extraction following the method as described in Pocasap *et al*. (2013). Fresh RS was blended with deionized water (1:1 ratio) for 30 mins. and left for autolyzing at room temperature for another 2 hrs. Then, the homogenate was filtered to collect filtrate for further liquid-liquid extraction with dichloromethane. The solvent was removed by rotary evaporator yielding the dry crude extract.

Antiproliferation assay

The antiproliferation of the extract against the cancer cell lines were determined by neutral red assay (Machana *et al*., 2011) in the human lung adenocarcinoma cells (SK-LU1),

human breast adenocarcinoma cells (MCF-7) and normal African green monkey kidney epithelial (Vero) cell lines. Briefly, cells density of $3x10^4$ cells/well were treated with RS extracts at various concentrations. After 24 hours of treatment, cells were washed with 1x PBS and added with NR solution (final concentration of 50 µg/ml). After 2 hours, cells were lysed by 0.33% HCl in isopro panol. The absorbance of NR was detected by a dual-wavelength UV spectrometer at 537/650 nm. A plot of % cytotoxicity versus concentrations of test compounds was used to extrapolate the concentration possessing 50% cytotoxicity (IC_{50}). The IC_{50} values in the normal cells versus that in the cancer cells was calculated to achieve the selective index (SI) value.

Statistical analysis

The results were expressed as mean ± standard deviation (SD) and the statistical differ ence between treatments were using a one-way analysis of variance (one-way ANOVA). The comparison of selective index between two cell lines was tested using one sample t-test. The *p*-values below 0.05 were considered statistically test significant at 95% confidential level using Duncan.

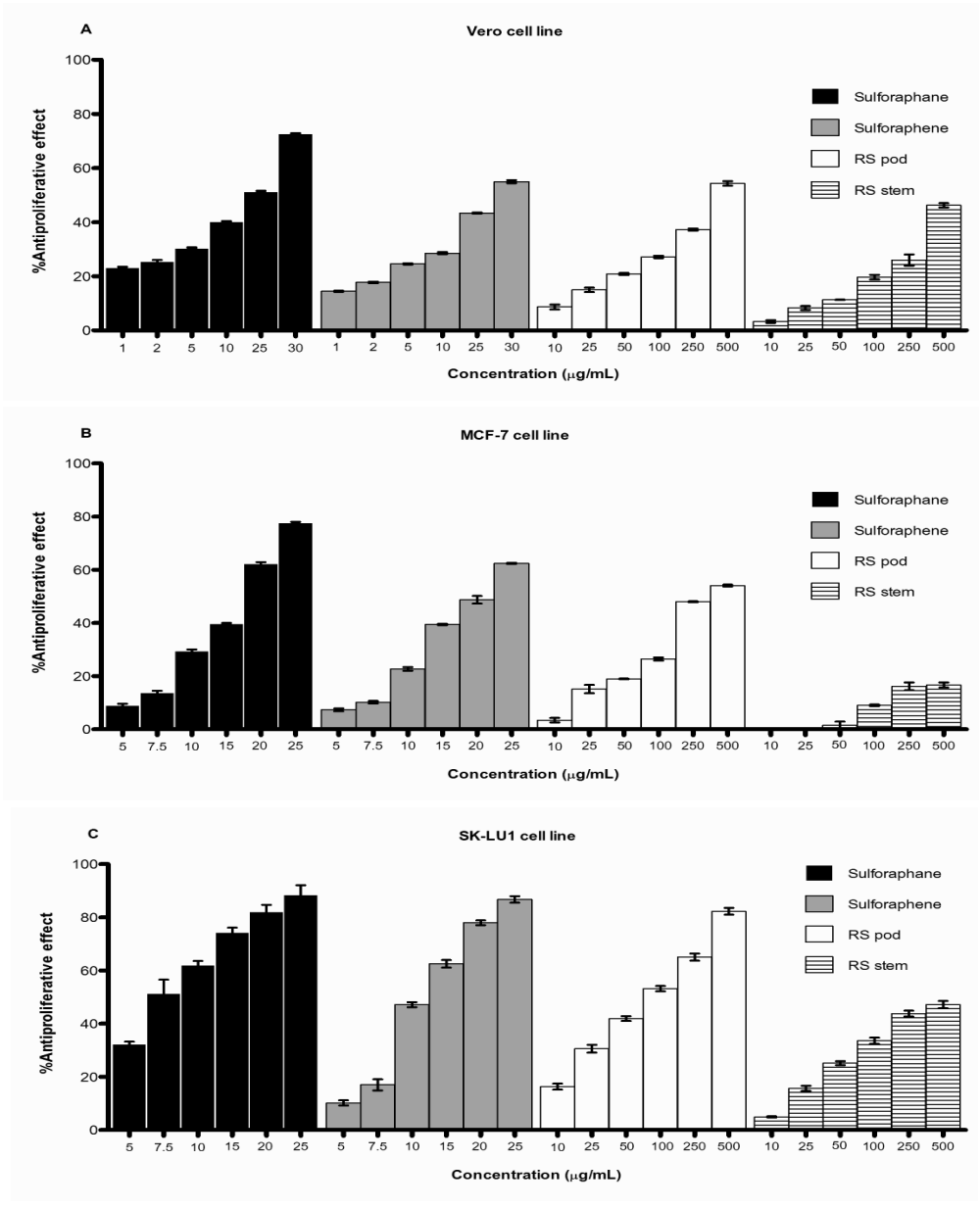

Figure 1 Antiproliferative effect of positive isothiocyanates standard compounds and Thai rat-tailed radish extracts.

Tables 1 Antiproliferative effect represented by an IC_{50} values of standard sulforaphane, sulfora phene, and the extracts from RS pod and stem in the human lung cancer (SK-LU1),the human breast cancer (MCF-7), and the normal Vero cell lines.

Test compounds	IC_{50} (μg/ml)		
	MCF-7	SK-LU1	Vero
Sulforaphane	$73.7\pm0.3^{a,C}$	$8.1 \pm 0.6^{a,A}$	$25.2\pm0.1^{a,B}$
Sulforaphene	$161.2 \pm 1.5^{b,C}$	$17.0 \pm 0.2^{b,A}$	$39.9\pm0.4^{b,B}$
RS pod	$391.6\pm1.8^{c,B}$	$84.2\pm2.4^{c,A}$	$427.2 \pm 4.9^{c,C}$
RS stem	inactive	inactive	inactive

Note IC_{50} values followed by different small superscript letter in the column share significant differences at 5% probability by one-way ANOVA test.

IC_{50} values followed by different capital superscript letter in the row share significant differences at 5% proba bility by one-way ANOVA test.

Inactive means less than 50% cytotoxicity achieved even when cells were treated with the maximum concen tration of 500 μg/ml.

Table 2 Selective index of test compounds in each cancer cell line and difference of selective index between MCF-7 and SK-LU1 represented as the p value.

Test compounds	***Selective index***		***p value***
	MCF-7	**SK-LU1**	
Sulforaphane	0.3 ± 0.0	3.1 ± 0.2	0.039
Sulforaphene	0.2 ± 0.0	2.3 ± 0.0	0.040
RS pod	1.1± 0.0	5.1± 0.1	0.018
RS stem	nd	nd	nd

Note: $p< 0.05$ significantly difference with 95% confidence level by t-test.

nd = cannot be determined.

3. Results

Table 1 illustrated the concentration causing 50% cytotoxicity to the cell (IC_{50}) of the test compounds. The SK-LU1 cell was more sensitive to the test compounds than normal Vero and MCF-7 cells, respectively. Sulforaphane was highly toxic against the SK-LU1 and MCF-7 cell lines with the IC_{50} values of 8.1 ±0.6 μg/ml and 73.7 ±0.3 μg/ml, respectively. The RS pod extract showed moderate cytotoxic against the SK-LU1 and MCF-7 cell lines with the IC_{50} values of 84.2±2.4 μg/ml and 391.6 ±1.8 μg/ml, respec tively.

In consideration of the selective cyto toxic based on the selective index, sulforaphane, sulforaphene, and RS pod extract were selec tively cytotoxic to SK-LU1 cancer cells (Table 2). Interestingly, only the RS pod extract possessed the highest selectivity in the SK-LU1 cells over the Vero cells. The RS pod extract was selective on both human carcinoma MCF-7 and SK-LU1cell lines comparable with the standard ITCs with the SI value of 1.1 and 5.1, respectively (p<0.05, one-way ANOVA,Duncan). It was previously reported that the selective index of the cytotoxic compound more than 3 is preferably considered to be anti-cancer selective compound (Machana *et al*., 2011).

This study was firstly reported the poten tial anticancer activity of Thai native vegetable—Thai rat-tailed radish—on the lung carcinoma SK-LU1. Notably, our presentstudy reports the antiproliferative effect of the edible pod part of RS.

4. Conclusion

The extraction of RS pod possessed selective antiproliferative effect against both lung and breast cancer cell lines—with the higher effective in lung cancer cell. The consuming of vegetables has been revealed not only the health benefits from fibers, vitamins, and minerals. Our study confirms rich phytochemical in the RS pod part. Therefore, RS was potential natural sources for developing health promoting or chemopreventive compound. Further study on the elucidation of attributed bioactive phytochemical and extended study on the anticancer activity are under inves-tigation.

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